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14. ABSTRACT
The chemical properties and high density of depleted uranium (DU) render the metal well suited for military purposes, but knowledge of DU neurotoxicity and its treatment is lacking. This project is designed to test the hypothesis that *long-term administration of a free radical trapping agent and/or an NMDA receptor antagonist will reduce neurotoxicity resulting from chronic exposure to DU.* This hypothesis is consistent with previous observations ensuing from chronic intramuscular DU pellet implants in rats, and is based on the anticipation that specific pharmacological agents will reverse signs of DU-induced oxidative stress. As prescribed by the Statement of Work, efforts were initiated in year 1 on Tasks 1 (drug therapies to reverse DU-induced elevations in extracellular glutamate) and 2 (brain DU concentrations) utilizing experimental groups (0, 300, and 600 mg DU) exposed for 9 months. Task 1 incorporates chronic neuroprotectant drug administration via implanted osmotic minipumps to address these objectives. Progress has been achieved on each Task, and remaining subject cohorts will be analyzed in year 2. Efforts have also begun to set up the biochemical assays to achieve Task 3 (biochemical markers of DU-induced oxidative stress in hippocampal tissue). Thus, progress is proceeding according to the schedule specified in the Statement of Work.

15. SUBJECT TERMS
depleted uranium, glutamate release, military disease, hippocampus, oxidative stress, neuroprotectant drugs

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Table of Contents

INTRODUCTION

The chemical properties and high density of depleted uranium (DU) render the metal well suited for military purposes. The U. S. Army utilizes DU for tank armor and in munitions, deployed such weapons in Gulf War I, and is currently deploying them in Iraq. However, knowledge of DU neurotoxicity and its treatment is lacking despite the apparent neurological basis of several components of Gulf War illness. Research in chronically exposed rats has reported alterations in hippocampal synaptic transmission, suggesting DU-induced decreases in neuronal excitability (1). This project is examining potential treatment options to address neurotoxicity from chronic DU exposure. On the basis of previous observations the bases of DU neurotoxicity are proposed to be cellular oxidative stress and the consequent increased production of reactive oxygen species, leading to decreased glutamate uptake and increased synaptic glutamate concentrations in conjunction with NMDA receptor up-regulation. Uranium-induced oxidative stress has previously been reported in rat kidney, testis, and lung (2-3). Studies will identify various biochemical markers of metal-induced oxidative stress in hippocampal tissue, and in combination with enhanced extracellular glutamate and NMDA receptor activity will provide three components of DU neurotoxicity for assessment of therapeutic efficacy. *It is hypothesized that long-term administration of a free radical trapping agent and/or an NMDA receptor antagonist will reduce DU neurotoxicity.* These studies will provide critical information on which to base new treatments for ill Gulf War veterans.

BODY

As prescribed in the approved Statement of Work, project activities in year 1 primarily addressed Tasks 1 and 2. A description of these efforts and the resulting progress toward each objective is provided below.

Task 1 concerns demonstration of the efficacy of chronically administered drug therapies to reverse DU-induced elevations in extracellular glutamate in superfused hippocampal slices from chronically exposed animals. The project includes a control group and low (300 mg load) and high dose (600 mg load) DU exposure conditions, but utilizes a vehicle and three drug-treated groups (memantine or riluzole or a combination) for each exposure level. This design results in a 3 exposure level × 4 drug condition matrix with 8 animals/cell (96 animals/cohort). This plan maximizes the ability to discriminate the actions of the therapeutic agents on the proposed measures. Drugs are administered via osmotic minipumps (Alzet) surgically inserted subcutaneously. Adult male Sprague-Dawley rats are implanted intramuscularly with DU pellets at 70-80 days of age; beginning at 3-4 months of age they are placed on food restriction so that their maximal body weight does not exceed 500-550 grams. After 7 months exposure 28-day minipumps are implanted and replaced once to cover the period up to 9 months when exposure is terminated and testing conducted. This is an appropriate interval for drug administration as the slope of the increase in DU concentration is greater during this period than prior to 6 months exposure. The minipumps are filled with drug solutions of 30 mg/ml memantine (3.6 mg/kg/day) and/or 10 mg/ml riluzole (1.2 mg/kg/day). Besides its potential usefulness as an uncompetitive NMDA receptor antagonist, memantine also has been reported to have neuroprotectant value via induction of brain-derived neurotrophic factor and its receptor (4-5), making the drug of particular interest for this project. Blood samples are being collected from

these animals to quantify plasma drug levels and validate the drug administration protocols. ^{3}H-Glutamate uptake into cortical slices from these same animals is being measured as a focused assessment of the integrity of the neurotransmitter transport process. Table 1 summarizes progress on this Task to date (~40% complete) by listing within the experimental design the numbers of animals that have completed exposure and drug treatments. Ultimately, each cell of the matrix will contain 8 animals.

Table 1

Number of Animals Completing Exposure and Testing

DU Exposure, mg pellets	Vehicle	Memantine	Riluzole	Memantine & Riluzole
0	4	3	3	3
300	3	4	3	3
600	2	3	4	3

Task 2 consists of determination of DU concentrations in brain tissue of chronically exposed animals at durations corresponding to the beginning (7 months) and end (9 months) of the drug therapies. Characterization of the protocol in this Task is necessary to give proper context to the experimental findings generated under the other Tasks. The determination of hippocampal uranium levels will be performed by inductively coupled plasma-mass spectrometry (ICP-MS) analysis by a commercial laboratory (Elemental Analysis, Inc., Lexington, KY). This methodology has proven more sensitive and reliable for this sample matrix than alternative approaches, and this vendor has previously provided reliable determinations. The DU used in this project consists of 30 mg pellets (1 mm diameter × 2 mm length) obtained from Aerojet Ordnance Tennessee (Jonesborough, TN), and are sterilized prior to use. Ten pellets are implanted in the gastrocnemius muscle of each thigh of 70-80 day old male rats. The design includes three exposure groups: a high dose group in which all pellets are DU (600 mg load), a low dose group receiving 10 pellets of DU (300 mg load), and a control group which received 20 tantalum pellets (0 mg load). The low dose group also receives 10 pellets of tantalum. Tantalum is an essentially inert heavy metal widely used in medical prostheses. The group size (N = 6) can result in relatively high coefficients of variation, but is sufficient to characterize the exposure protocol and provide general measures of metal uptake. At this point in time all tissue samples from the 7 month exposure groups have been harvested, and hippocampi from the 9 month groups will be collected shortly. All tissues will be processed by the analytical laboratory in one batch.

Task 3 concerns assessments of biochemical markers of DU-induced oxidative stress in hippocampal tissue and the ability of drug therapies to reverse the changes in these measures, and work has begun to set up these assays. The determinations will utilize commercially available kits, and analyses of more meaningful markers – e.g, F_2-isoprostanes – may be performed in conjunction with the markers originally proposed – catalase and glutathione

peroxidase activities and tissue non-protein hydroperoxides. DU pellet implantations in this cohort of animals will begin shortly.

Some problems have been encountered in the first year of the project. One of the drugs proposed as therapy – NXY-059 (Cerovive) – was no longer available from the manufacturer for experimental use, and thus had to be replaced in the experimental design. Riluzole inhibits Na^{+} and Ca^{+2} channel currents and attenuates synaptic glutamate release (6-7), and thus possesses neuroprotective and antioxidant capabilities. This drug is readily available, has been previously administered via osmotic minipumps, and thus constitutes a suitable alternative. Another challenge was the purchase of sufficient DU pellets (3000+ 30 mg pieces) to cover needs for the entire project, as this would result in the most economical unit price. Because of the high cost and amount ordered, there was a 2-3 month delay in filling the order, causing a delay in initiating Task 1. Finally, both members of Dr. Lasley's laboratory support staff were new hires and had to be trained on all aspects of the investigation, resulting in further initial delays. However, all these problems have been addressed, and the project is now progressing in a forthright manner.

KEY RESEARCH ACCOMPLISHMENTS

Considerable effort has been invested to optimize the surgical procedures for DU pellet and osmotic minipump implants, particularly since the latter had to be replaced once in each animal at the 8 month exposure interval. The consistency and reliability of the drug administration regimen is critically important in being able to demonstrate a neuroprotective/antioxidant effect for memantine and/or riluzole, and plasma drug determinations will support the other neurochemical tests to be conducted.

Because of the long DU exposure duration and projections that Tasks 1 and 2 would not be completed until the second year of the investigation, there are no meaningful results to be reported at this time. Nonetheless, the project is progressing according to schedule.

REPORTABLE OUTCOMES

None at this time.

CONCLUSIONS

Summaries of the progress and its importance as a scientific product are included in the preceding sections **Key Research Accomplishments** and also in **Body**. No conclusions on the effectiveness of memantine and/or riluzole to reverse the effects of DU-induced neurotoxicity can be stated at this time. The chronic DU exposure and drug administration protocols will be established by analytical determinations to be conducted in the coming months.

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APPENDICES

None

SUPPORTING DATA

None